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Effect of pyrimethanil on *Cryptococcus laurentii*, *Rhodosporidium paludigenum*, and *Rhodotorula glutinis* biocontrol of *Penicillium expansum* infection in pear fruit

Chen Yu, Tao Zhou, Kuang Sheng, Lizhen Zeng, Changzhou Ye, Ting Yu *, Xiaodong Zheng **

Department of Food Science and Nutrition, Zhejiang University, Hangzhou 310058, People's Republic of China

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ABSTRACT

The effect of biocontrol yeasts and pyrimethanil at low concentration on inhibition of blue mold rot caused by *Penicillium expansum* in pear fruit was investigated. Pyrimethanil at low concentration (40 μg/mL) alone had little inhibitory activity against the *P. expansum* infection in pear fruit wounds although it was effective in inhibiting the survival of *P. expansum* on Asp-agar medium. Pyrimethanil at this low concentration significantly enhanced the efficacy of *Cryptococcus laurentii* at 1×10^7 CFU/mL in reducing blue mold rot *in vivo* compared with *C. laurentii* at 1×10^7 CFU/mL alone. However, there was no additive inhibitory activity when pyrimethanil was combined for application with biocontrol yeasts *Rhodosporidium paludigenum* or *Rhodotorula glutinis*. Combination of pyrimethanil and *C. laurentii* at low concentration also inhibited blue mold rot when *P. expansum* was inoculated into fruit wounds 12 h before treatment and fruit was stored at low temperature (4 °C). Pyrimethanil at 0.04 to 400 μg/mL did not influence the survival of *C. laurentii in vitro*, and it only slightly reduced the population growth of *C. laurentii* after 48 h of incubation in the pear fruit wounds. There was no significant difference in quality parameters including total soluble solids, titratable acidity and ascorbic acid of pear fruit wounds among all treatments after 5 days of treatment at 25 °C. Integration of *C. laurentii* and pyrimethanil at low concentration might be an effective and safe strategy to control *P. expansum* infection in pear fruit, especially in an integrated postharvest disease management strategy.

1. Introduction

Blue mold rot caused by *Penicillium expansum* Link is one of the major postharvest diseases of pear fruit world-wide (Rosenberger, 1990). Fungicide application is the most effective method to control postharvest mold rots of pear (Eckert and Sommer, 1967) and other fruit (Forster et al., 2007; Hao et al., 2010; Koh et al., 2005; Prusky et al., 2006). However, postharvest treatments with fungicides are increasingly being limited because of environmental and toxicological risks as well as the onset of fungicide-resistant strains of fungal pathogens (Lima et al., 2011). Therefore, great efforts have been made to exploit alternatives to synthetic chemicals in the past 20 years (Droby et al., 2009).

Strains of *Cryptococcus laurentii* are biocontrol yeasts which have been widely studied and shown antagonistic activity in reduction of mold rot in pear fruit when applied alone or with various additives, including low dose of the traditional fungicide thiabendazole (Benbow and Sugar, 1999; Lima et al., 1998; Roberts, 1990; Sugar and Spotts, 1999; Yao et al., 2004).

Pyrimethanil belongs to the anilinopyrimidine class of fungicides, which has been registered for postharvest application to pears in the United States since 2004 (Kanetis et al., 2007; Sugar and Basile, 2008, 2011; Xiao and Boal, 2009). The antifungal activity of pyrimethanil *in vitro* could result from a block in the excretion of hydrolytic enzymes involved in the pathogenesis in a site-specific manner and/or from an inhibition of methionine biosynthesis in fungal cells (Leroux and Gredt, 1996). This fungicide is highly effective in inhibiting conidial germination and germ-tube elongation of *P. expansum* (Li and Xiao, 2008a). Currently, pyrimethanil is increasingly being used as an alternative to thiabendazole for control of blue mold and other postharvest diseases in pome fruits, and particularly as a tool for control of blue mold caused by thiabendazole resistant strains of *P. expansum* (Li and Xiao, 2008a). However, to our knowledge, the sensitivity of antagonistic yeasts to pyrimethanil and the impacts of pyrimethanil on the efficacy of antagonistic yeasts have not been explored.

The objective of this study was to investigate the effect of pyrimethanil on inhibition of *P. expansum* and *C. laurentii in vitro* and *in vivo* and the potential of the combined use of pyrimethanil and *C. laurentii* in reducing the postharvest blue mold rots of pear fruit.

2. Materials and methods

2.1. Microorganisms and material

Pear fruit (*Pyrus pyrifolia* Nakai. cultivar "Shuijing") were selected for uniform size, ripeness and absence of mechanical damage. After

* Corresponding author. Tel.: +86 571 88982398; fax: +86 571 88982191.
** Corresponding author. Tel.: +86 571 88982861; fax: +86 571 88982191.
E-mail addresses: yuting@zju.edu.cn (T. Yu), xdzheng@zju.edu.cn (X. Zheng).

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being immersed in a solution of 0.1% sodium hypochlorite (actual concentration of available chlorine ≥ 52 μg/mL) for about 1–2 min, fruit were washed with tap water and were allowed to air dry at the room temperature (approximately 25 °C).

The yeast *C. laurentii* (Kufferath) Skinner was isolated from the surface of pear fruit, *Rhodosporidium paludigenum* Fell & Tallman was originally isolated from the south East China Sea, *Rhodotorula glutinis* (Fresenins) Harrison was obtained from the Institute of Microbiology, Chinese Academy of Science (Beijing, P.R. China), respectively. Previously these yeasts had been reported to have biocontrol activity against postharvest fungal diseases (Wang et al., 2008; Yu et al., 2007; Zheng et al., 2005). The culture of biocontrol yeasts and the preparation of their cell suspensions were carried out as previously reported (Wang et al., 2008; Yu et al., 2007; Zheng et al., 2005). The yeasts were cultured in 250 mL conical flasks containing 50 mL of nutrient yeast dextrose broth (NYDB, containing 8 g nutrient broth, 5 g yeast extract, and 10 g glucose in 1 L of distilled water) medium at 28 °C for 48 h on a rotary shaker at 200 rpm. Yeast cells were centrifuged at 3000 rpm for 10 min and washed twice to remove the growth medium. The yeasts were resuspended with sterile distilled water and adjusted to required concentration.

P. expansum was originally isolated from infected pear fruit and cultured on potato-dextrose agar (PDA) medium (containing the extract from 200 g potato, 20 g glucose and 20 g agar in 1 L of distilled water) at 25 °C in the dark. Spore suspensions were obtained by flooding 7-day-old culture medium of *P. expansum* with sterile distilled water. The number of spores was calculated with the aid of a hemacytometer and the spore concentration was adjusted with sterile distilled water as required.

Pyrimethanil (as Scala, 40%, active ingredient) was obtained from BayerCropScience, China.

2.2. Efficacy of antagonistic yeasts and pyrimethanil in reducing blue mold rot on pear fruit wounds

2.2.1. Effect of different antagonistic yeasts and pyrimethanil on blue mold rot

Wounds were made (5 mm diameter and 3 mm deep) on each pear fruit with the tip of a sterile borer and each wound was treated with 50 μL of one of the following: (1) pyrimethanil at 400 μg/mL of active ingredient (the maximum label rate), pyrimethanil at 40 μg/mL of active ingredient, (2) cell suspensions of *C. laurentii* at 1×10^8 CFU/mL, (3) cell suspensions of *C. laurentii* at 1×10^7 CFU/mL alone or with pyrimethanil in final concentration at 40 μg/mL of active ingredient, (4) cell suspensions of *R. paludigenum* at 1×10^7 CFU/mL alone or with pyrimethanil in final concentration at 40 μg/mL of active ingredient, (5) cell suspensions of *R. glutinis* at 1×10^7 CFU/mL alone or with pyrimethanil in final concentration at 40 μg/mL of active ingredient and (6) sterile distilled water as control. Two hours later, 30 μL of *P. expansum* spore suspension at 1×10^4 spores/mL was inoculated into each wound. The pears were then air dried and stored in the covered plastic trays to maintain a 90% relative humidity at 25 °C. The percentage of infected fruit wounds and the average lesion diameters of the treated fruit were examined daily after inoculation.There were three replicates per treatment with six pears per replicate. The data are from one individual experiment and are representative of two independent experiments with similar results.

2.2.2. Effect of time between biocontrol treatment and pathogen inoculation on blue mold rot

Four wounds were made on each pear fruit as above and inoculated with 30 μL of *P. expansum* spore suspension at 1×10^4 spores/mL. Twelve hours later, each fruit wound was treated with 50 μL of the one of the following: (1) sterile distilled water as the control; (2) pyrimethanil at 40 μg/mL of active ingredient; (3) *C. laurentii* at 1×10^7 CFU/mL; (4) *C. laurentii* at 1×10^7 CFU/mL plus pyrimethanil in final concentration at 40 μg/mL of active ingredient. The pears were then air dried and stored in the covered plastic trays to maintain a 90% relative humidity at 25 °C. The percentage of infected fruit wounds and the average lesion diameters of the treated fruit were examined daily after inoculation. There were three replicates per treatment with six pears per replicate. The data are from one individual experiment and are representative of two independent experiments with similar results.

2.2.3. Effect of C. laurentii and pyrimethanil on blue mold rot at low temperature

Six wounds were made on each pear fruit as above and each wound was treated with 50 μL of one of the following: (1) sterile distilled water as the control; (2) *C. laurentii* at 1×10^8 CFU/mL; (3) pyrimethanil at 400 μg/mL of active ingredient; (4) pyrimethanil at 40 μg/mL of active ingredient; (5) *C. laurentii* at 1×10^7 CFU/mL; (6) *C. laurentii* at 1×10^7 CFU/mL + pyrimethanil in final concentration at 40 μg/mL of active ingredient. Two hours later, 30 μL of *P. expansum* spore suspension at 1×10^4 spores/mL was inoculated into each wound. The pears were then air dried and stored in the covered plastic trays to maintain a high relative humidity at 4 °C. The percentage of infected fruit wounds and the average lesion diameters of the treated fruit were examined daily after inoculation. There were three replicates per treatment with six pears per replicate. The data are from one individual experiment and are representative of two independent experiments with similar results.

In the above three experiments, when the visible rot zone beyond the wound area on each fruit was more than 1 mm wide, it was counted as an infected fruit and lesion diameter included wound diameter. Disease incidence and lesion diameter were measured according to the following formulas:

$$\text{Disease incidence } (\%) = \frac{\Sigma \text{ Numeber of infected pear fruit}}{\text{Total number of treated fruit}} \times 100$$

$$\text{Lesion diameter } (\text{mm}) = \frac{\Sigma \text{ Lesion diameter of infected pear fruit}}{\text{Total number of treated fruit}}$$

2.3. In vitro effect of pyrimethanil on the colonization of P. expansum and C. laurentii

The effect of pyrimethanil on the colonization of *P. expansum* and *C. laurentii* was determined on Anilinopyrimidines (Aps) agar medium (1 g K_2HPO_4 and 1 g $MgSO_4 \cdot 7H_2O$ were each dissolved in 30 mL water (stocks I and II), 0.5 g KC1 and 0.01 g $FeSO_4 \cdot 7H_2O$ were dissolved in 40 mL distilled water (stock III), 2 g Lasparagine and 15 g agar were dissolved in 400 mL water (stock IV) and 22 g glucose$\cdot H_2O$ was dissolved in 490 mL water (stock V)). Stocks I and II were pooled. The precipitate that was formed was dissolved by adding 10 M hydrochloric acid dropwise, and then stock III was added. Again, the formation of a precipitate was observed, which dissolved after adding stock IV and autoclaving at 1.013 bar for 20 min. Stock V was autoclaved separately and then pooled with the rest. The pH values of all components ranged between 6.5 and 7.0. Pyrimethanil was dissolved in acetone before mixing with the agar that was cooled to 50 °C according to the method described by Hilber and Schuepp (1996). Pyrimethanil was added to the Aps-agar medium at the final concentration of 0, 0.04, 0.4, 4, 40 and 400 μg/mL of active ingredient, respectively. The concentration of yeast cells or the pathogen spores was diluted to 1×10^3 yeast CFU/mL or the pathogen spores/mL with sterile distilled water and 100 μL of each suspension was spread on Asp-agar. After the plates were incubated for 48 h–72 h at 28 °C, the colonies per plate were counted and the results are expressed as the mean number of colony forming units (CFU) per plate. Each treatment was replicated three times with three plates per replicate. The data are from

one individual experiment and are representative of two independent experiments with similar results.

2.4. Effect of pyrimethanil on the colonization of C. laurentii in pear fruit wounds

Three wounds were made on each pear fruit as above. Then, the wounds were treated with 50 μL *C. laurentii* cell suspension (approximately 1×10^7 CFU/mL) alone or with pyrimethanil in final concentration at 40 or 400 μg/mL of active ingredient. The samples were then stored at 25 °C and taken at different times (0, 24, 48, and 72 h) after inoculation. The tissue was removed with a sterile borer (1 cm diameter and 1 cm deep) and ground with a mortar and pestle in 10 mL of sterile water. Then, 100 μL of serial 10-fold dilutions were spread on NYDA (containing 8 g nutrient broth, 5 g yeast extract, 10 g glucose and 20 g agar in 1 L of distilled water) agar plates. Samples were taken within 2 h after treatment served as time 0. Colonies were counted after incubation at 25 °C for 2 days and expressed as the Lg CFU per wound. There were three replicates per treatment with three fruits per replicate. The data are from one individual experiment and are representative of two independent experiments with similar results.

2.5. Effect of C. laurentii and pyrimethanil on quality parameters of pear fruit

Five wounds were made on each pear fruit as above. Each wound was treated with 50 μL of one of the following: (1) sterile distilled water as the control; (2) pyrimethanil at 400 μg/mL of active ingredient; (3) pyrimethanil at 40 μg/mL of active ingredient; (4) *C. laurentii* at 1×10^7 CFU/mL; (5) *C. laurentii* at 1×10^7 CFU/mL with pyrimethanil in final concentration at 40 μg/mL of active ingredient. Fruit wounds were taken at 0 and 5 days after the treatment at 25 °C. Total soluble solids (TSS) content was determined by using a portable refractometer (WZ-103, Top Instrument Co. Ltd., China) and the results were expressed as percentages. Titratable acidity (TA) content was measured by titrating 10 mL of the filtered liquid to pH 8.1 with 0.01 M NaOH and calculating the result as % malic acid. Ascorbic acid (AA) content was determined using the 2, 6-dichloro-indophenol method and the results were expressed as mg/100 g. Each treatment was replicated three times with three fruits per replicate. The data are from one individual experiment and are representative of two independent experiments with similar results.

2.6. Statistical analyses

All treatments are arranged in a randomized complete block design and conducted at least twice. The data are from one individual experiment and are representative of two independent experiments with similar results. The data were analyzed on analysis of the variance (ANOVA) in the Statistical Program (SPSS/PC ver. 11.5, SPSS Inc. Chicago, Illinois, USA). When the analysis was statistically significant at the level $P < 0.05$, the Duncan's multiple range test was applied to separate the means. The data for each experiment were analyzed separately.

3. Results

3.1. Efficacy of antagonistic yeasts and pyrimethanil in reducing blue mold rot on pear fruit wounds

Pyrimethanil and *C. laurentii* inhibited the *P. expansum* infection in pear fruit wounds, which was positively correlated with the concentration of pyrimethanil and *C. laurentii* (Fig. 1). When the concentration of pyrimethanil was at 400 μg/mL or *C. laurentii* at 10^8 CFU/mL, blue mold rot was markedly inhibited after 4 days of inoculation with *P. expansum* at 25 °C. However, the efficacy was greatly reduced when the concentration of pyrimethanil and *C. laurentii* was 40 μg/mL or 10^7 CFU/mL. Combining pyrimethanil at 40 μg/mL with *C. laurentii* at 1×10^7 CFU/mL resulted in greater inhibition of the blue mold rot than pyrimethanil at 40 μg/mL or *C. laurentii* at 1×10^7 CFU/mL used alone. The co-treatment of pyrimethanil at 40 μg/mL with *C. laurentii* at 1×10^7 CFU/mL was similar to that of pyrimethanil at concentration of 400 μg/mL or *C. laurentii* at 1×10^8 CFU/mL at significance level. However, when pyrimethanil was added to the suspension of *R. paludigenum* or *R. glutinis*, disease inhibition was not enhanced compared with the treatment with *R. paludigenum* or *R. glutinis* alone.

When *P. expansum* was inoculated into the fruit wounds 12 h before treatment, the combination of *C. laurentii* at 1×10^7 CFU/mL and pyrimethanil at 40 μg/mL exhibited a stronger inhibition than the treatment with *C. laurentii* at 1×10^7 CFU/mL and pyrimethanil at 40 μg/mL alone (Fig. 2).

On condition that *P. expansum* was inoculated into the fruit wounds 2 h after treatment, the biocontrol efficacy of *C. laurentii* at 1×10^7 CFU/mL with pyrimethanil in final concentration at 40 μg/mL was also significantly higher than *C. laurentii* at 1×10^7 CFU/mL or pyrimethanil at 40 μg/mL used alone after 16 days of inoculation with *P. expansum* at 4 °C (Fig. 3), which was similar to the efficacy of *C. laurentii* used at high concentration (1×10^8 CFU/mL).

3.2. In vitro effect of pyrimethanil on the colonization of P. expansum and C. laurentii

Pyrimethanil at 0.04 μg/mL to 400 μg/mL had little effect on the survival of *C. laurentii* on Aps-agar medium (Table 1). Pyrimethanil at 0.04 μg/mL to 4 μg/mL did not influence the survival of *P. expansum* on Asp-agar medium after 48 h–72 h of inoculation. However, when the concentration was increased to 40 μg/mL or higher, it inhibited the survival of *P. expansum* entirely.

3.3. Effect of pyrimethanil on the colonization of C. laurentii in pear fruit wounds

C. laurentii proliferated rapidly in pear fruit wounds, especially during the first 24 h of the incubation and the population of the antagonist continued to increase during 24 to 48 h and thereafter dropped slightly during 48 to 72 h (Fig. 4). There was no significant difference of the colonization of *C. laurentii* in the presence of pyrimethanil from 40 to 400 μg/mL of active ingredient during the first 24 h of incubation. However, pyrimethanil inhibited the increase of the yeast population after 48 h of incubation and there was a significant lower population of the treatment with pyrimethanil than that of the control at 48 h. Afterwards, the colonization of *C. laurentii* was similar among different treatments at 72 h.

3.4. Effect of C. laurentii and pyrimethanil on quality parameters of pear fruit

There was no significant difference in quality parameters including TSS, TA and AA of pear fruit wounds among all treatments after 5 days of treatment at 25 °C (data not shown).

4. Discussion

Pyrimethanil at 400 μg/mL (the maximum label rate) exhibited a strong inhibition of *P. expansum in vitro* and *in vivo*, which was consistent with the results reported by Li and Xiao (2008a), Sholberg et al. (2005), Sugar and Basile (2008) and Xiao and Boal (2009). However, pyrimethanil failed to reduce *P. expansum* infection in pear fruit wounds when its concentration was 40 μg/mL although pyrimethanil at this concentration inhibited the pathogen *in vitro*, indicating that

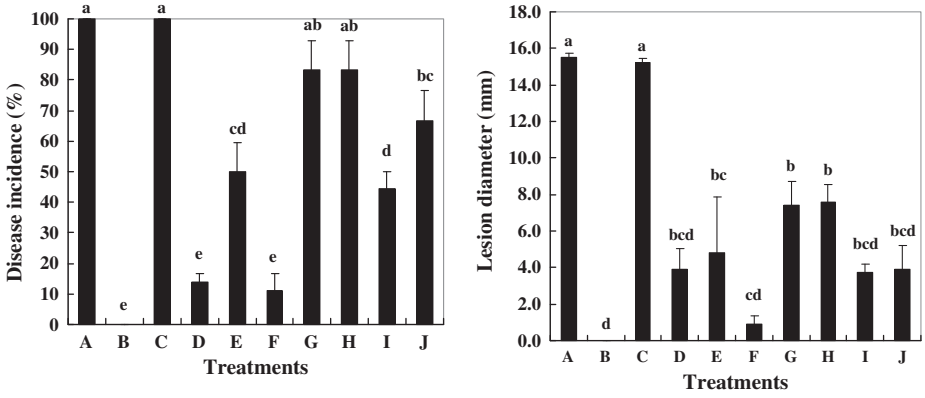

Fig. 1. Inhibitory activity of biocontrol yeasts and pyrimethanil on blue mold rot in pear fruit wounds after 4 days of inoculation with *Penicillium expansum* at 25 °C. Each fruit wound was treated with the following agent and then inoculated with *Penicillium expansum* within 2 h. A: Sterile distilled water as control; B: pyrimethanil at 400 μg/mL; C: pyrimethanil at 40 μg/mL; D: *Cryptococcus laurentii* at 1×10^8 CFU/mL; E: *Cryptococcus laurentii* at 1×10^7 CFU/mL; F: *C. laurentii* at 1×10^7 CFU/mL + pyrimethanil at 40 μg/mL;G: *Rhodosporidium paludigenum* at 1×10^7 CFU/mL; H: *Rhodosporidium paludigenum* at 1×10^7 CFU/mL + pyrimethanil at 40 μg/mL; I: *Rhodotorula glutinis* at 1×10^7 CFU/mL; J: *Rhodotorula glutinis* at 1×10^7 CFU/mL + pyrimethanil at 40 μg/mL. Each value is the mean of three separate determinations and bars represent the standard errors. Different letter indicates significant differences ($P = 0.05$) according to the Duncan's multiple range test.

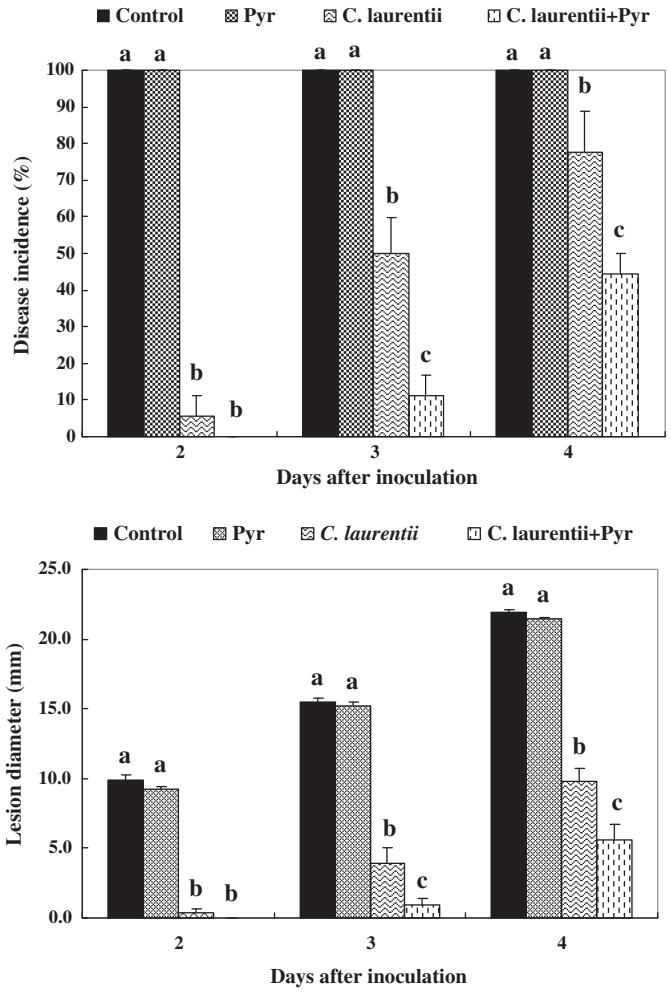

Fig. 2. Inhibitory activity of *Cryptococcus laurentii* and pyrimethanil on blue mold rot in pear fruit wounds after 2, 3 and 4 days of inoculation with *Penicillium expansum* at 25 °C. Each fruit wound was inoculated with *P. expansum* 12 h before treatment and then treated with the following agent. Sterile distilled water as control; pyrimethanil at 40 μg/mL; *C. laurentii* at 1×10^7 CFU/mL; *C. laurentii* at 1×10^7 CFU/mL + pyrimethanil at 40 μg/mL. Each value is the mean of three separate determinations and bars represent the standard errors. Different letter indicates significant differences ($P = 0.05$) according to the Duncan's multiple range test for each time point.

the results from the *in vitro* testing cannot always accurately represent the efficacy of that *in vivo* situations (Rotem et al., 1978).

Biological control with yeast antagonists has attracted great interest as a promising way to control postharvest diseases in recent two

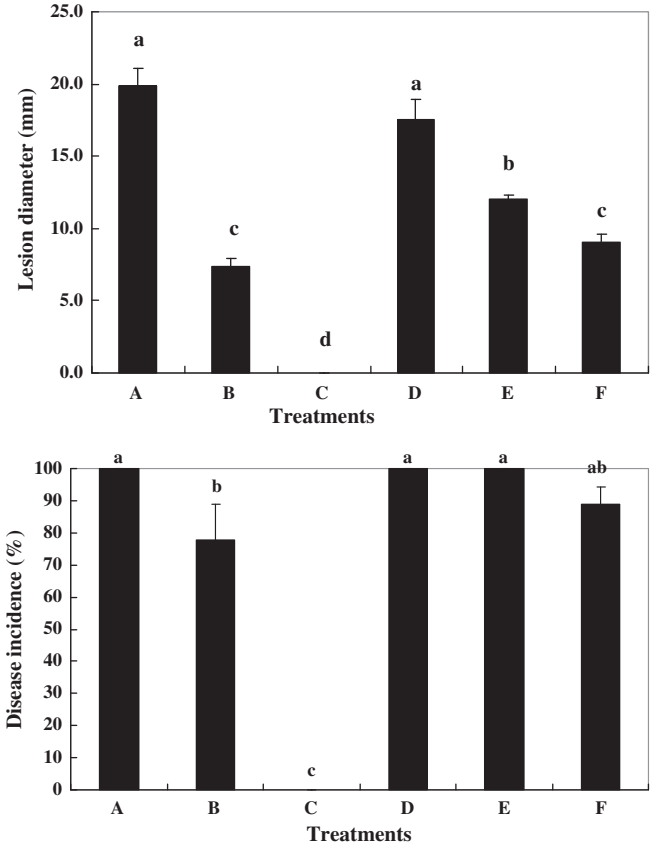

Fig. 3. Inhibitory activity of *Cryptococcus laurentii* and pyrimethanil on blue mold rot in pear fruit wounds after 16 days of inoculation with *Penicillium expansum* at 4 °C. Each fruit wound was treated with the following agent and then inoculated with *P. expansum* within 2 h. A: Sterile distilled water as control; B: *C. laurentii* at 1×10^8 CFU/mL; C: pyrimethanil at 400 μg/mL; D: pyrimethanil at 40 μg/mL; E: *C. laurentii* at 1×10^7 CFU/mL; F: *C. laurentii* at 1×10^7 CFU/mL + pyrimethanil at 40 μg/mL. Each value is the mean of three separate determinations and bars represent the standard errors. Different letter indicates significant differences ($P = 0.05$) according to the Duncan's multiple range test.

Table 1
In vitro effect of pyrimethanil on the colonization of *Cryptococcus laurentii* and *Penicillium expansum*.[a]

Treatment	*C. laurentii* on Asp-agar (CFU/plate)		*P. expansum* on Asp-agar (CFU/plate)	
Water control	34.3 ± 6.2	a	48.0 ± 2.3	A
Pyrimethanil at 0.04 μg/mL	33.7 ± 0.7	a	56.3 ± 8.1	a
Pyrimethanil at 0.4 μg/mL	38.3 ± 1.2	a	56.0 ± 6.4	a
Pyrimethanil at 4 μg/mL	32.0 ± 2.6	a	59.3 ± 8.3	a
Pyrimethanil at 40 μg/mL	36.0 ± 4.6	a	0.0 ± 0.0	b
Pyrimethanil at 400 μg/mL	29.7 ± 2.0	a	0.0 ± 0.0	b

[a] Each value is the mean of three separate determinations and the standard error. Different letters indicate significant differences ($P = 0.05$) according to one-way analysis of Duncan's multiple range test and the analysis for each microorganism was separated.

decades (Droby et al., 2009). However, microbial antagonists when applied alone usually do not bring about 100% control of postharvest diseases of fruits and vegetables (Sharma et al., 2009). Therefore, the effectiveness of a biocontrol agent must be improved so as to be developed into a really economically feasible alternative. It has been reported that the enhanced efficiency of yeast antagonist was achieved when the antagonist was in combined application with reduced level of fungicide such as imazalil, thiobendazole, boscalid, and cyprodinil (Lima et al., 2011; Sharma et al., 2009). However, to the best of our knowledge, there was no information regarding the combined efficacy of pyrimethanil and *C. laurentii* in inhibiting *P. expansum* infection in fruit.

Pyrimethanil from 40 μg/mL to 400 μg/mL had little adverse effect on the growth of the antagonist *in vitro* and *in vivo* overall, except for an inhibition on the population of *C. laurentii* after 48 h of incubation in pear fruit wounds, suggesting that the antagonist *C. laurentii* might be compatible with this fungicide even at the relative high concentrations. The biological activity of the antagonist is based on its capacity of rapid colonization in the fruit wounds, which is critical for its effective decay control. It has been reported that the biocontrol efficacy of *C. laurentii* was significantly reduced when its growth was inhibited by salicylic acid and indole-3-acetic acid (Yu and Zheng, 2006; Yu et al., 2009). Thus, it is important to maintain the colonization growth of biocontrol yeast when integrated with other disease control methods (Lima et al., 2006, 2011; Yu and Zheng, 2006; Yu et al., 2009).

Inherent characteristics of both pathogen and fungicide contribute to the development of resistant populations of a fungal pathogen to a fungicide. Pyrimethanil are considered medium-risk resistance fungicides, but pathogens such as *P. expansum* and *B. cinerea*, because of their short life cycles and abundant reproduction have the propensity to acquire fungicide resistance rapidly and are high-risk pathogens for the development of fungicide resistance (Brent and Hollomon, 1998).

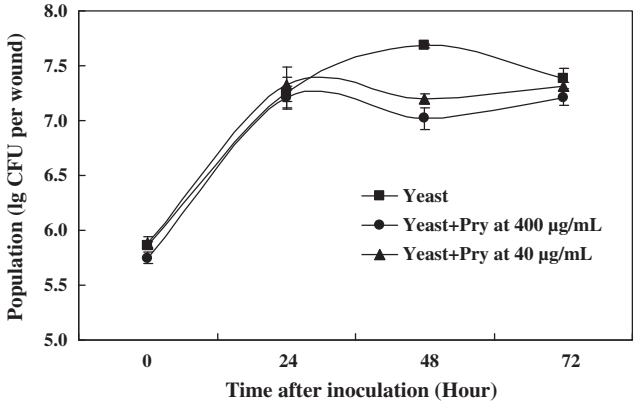

Fig. 4. Effect of pyrimethanil on the population of *Cryptococcus laurentii* in pear fruit wounds. Each value is the mean of three separate determinations and bars represent the standard errors.

Using a mutagenesis approach, some results indicated that pyrimethanil represented a higher risk than fludioxonil in the development of resistance in *P. expansum*, and that triple resistance to TBZ, fludioxonil, and pyrimethanil could develop and become a practical problem if pathogen populations develop resistance to pyrimethanil (Li and Xiao, 2008b).

The combination of *C. laurentii* and pyrimethanil at the low concentration would cause a greater inhibition of blue mold rot than application of *C. laurentii* or pyrimethanil at the low concentration alone, which was likely the result from the interplay of the antifungal property of pyrimethanil and the antagonistic activity of biocontrol yeast. This integrated treatment could not only reduce the dosage of fungicide and yeast, thus helping to minimize the fungicide residue and cost of biocontrol treatment, but also contributing to management of fungicide resistance (Lima et al., 2006, 2011).

Commercial applications of pyrimethanil usually ranged from 250 μg/mL to 1000 μg/mL (Li and Xiao, 2008a; Sholberg et al., 2005; Sugar and Basile, 2008; Xiao and Boal, 2009). Xiao and Boal (2009) reported that after treatment of apple with pyrimethanil at 0.5 mL/L (Penbotec 400SC at 1.25 mL/L), the residues of the fungicide pyrimethanil in apple fruit could persist for 5–7 months (about 2–3 μg/mL), which is below the maximum residue level (MRL) standard of pyrimethanil for pome fruit established by US-EPA (14 μg/mL), as well as the MRL proposed by Codex (7 μg/mL) and the Canadian government (3 μg/mL) (US-EPA, 2008). In this present study, the treatment concentration of pyrimethanil could be reduced to 40 μg/mL when combined with the biocontrol yeast *C. laurentii*; therefore, the residues of pyrimethanil would be certainly decreased further.

Integration of *C. laurentii* and pyrimethanil at low concentration might be an effective and safe strategy in field application to control *P. expansum* infection in pear fruit. However, further experiments should be conducted on other strains of *P. expansum* and at a higher scale with more fruits and with natural inoculum in order to confirm the inhibitory effect observed using a combination of *C. laurentii* and reduced concentration of pyrimethanil.

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